Ann. Hum. Genet., Lond. (1962), **26**, 107
Printed in Great Britain

Genetical linkage between the loci for glucose-6-phosphate dehydrogenase deficiency and colour-blindness in American Negroes

BY IAN H. PORTER,* JANE SCHULZE AND VICTOR A. MCKUSICK

From Division of Medical Genetics, The Department of Medicine, Johns Hopkins Hospital University, School of Medicine, Baltimore, Maryland, U.S.A.

Glucose-6-phosphate dehydrogenase (G-6-PD) deficiency is an inborn error of metabolism. It leads to reduction of red cell survival time when an affected person is exposed to certain drugs such as 8-amino-quinolines, sulphonamides, nitrofurantoin, phenacetin and others, fava beans and perhaps some virus infections (Marks, 1959).

As early as 1931 a racial difference in sensitivity to plasmaquine was noticed by Manifold, and in 1948 Turchetti drew attention to the familial nature of primaquine sensitivity and its similarity to favism. Hockwald, Arnold, Clayman & Alving (1952) showed that the incidence of primaquine sensitivity in American Negro males was 10%. The following year it was demonstrated that many drug-induced haemolytic anaemias were due to an intrinsic abnormality of the erythrocytes (Dern, Weinstein, Le Roy, Talmage & Alving, 1954), and in 1956 Carson, Flanagan, Ickes & Alving showed that this is due to a deficiency of the enzyme G-6-PD.

Limitation of the deficiency to certain racial groups suggested that G-6-PD deficiency had a genetic origin, and this was confirmed by Childs, Zinkham, Browne, Kimbo & Torbert (1958), who showed that it is probably *X*-linked, although they could not exclude sex-limited autosomal inheritance.

X-linkage can be distinguished from autosomal dominant inheritance with manifestation limited to the male by showing:

(1) that affected males married to non-carrier females never have affected male children, and if the trait is dominant, that all their female children are affected;

(2) that there is genetical linkage between the relevant locus and a marker locus, proven to be *X*-linked, such as colour-blindness; or

(3) that individuals of the chromosome constitution *XO* (Turner's Syndrome) have the same phenotype frequencies as males (Polani, Lessof & Bishop, 1956; Nilsson, Bergman, Reitalu & Waldenström, 1959; Penrose, 1961; Gartler, Vullo & Gardini, 1962).

Further, it has been shown that affected Negro and Caucasian males differ quantitatively in the expression of G-6-PD deficiency (Marks & Gross, 1959). Thus in contrast to affected Negro males, who have normal or near normal G-6-PD levels in the leukocytes and a mean erythrocyte enzyme activity 20% of normal, affected Caucasian males appear to have a leukocyte G-6-PD level about 30% of normal and red cell enzyme activity which averages 5% of normal values. It would, therefore, be interesting to find that the recombination fraction between the loci for G-6-PD deficiency and colour-blindness were also significantly different in the two ethnic groups, as this would be evidence that the difference in expression of G-6-PD deficiency is due to different genetical loci.

* Present address: Department of Medicine, Postgraduate Medical School, Hammersmith Hospital, London, W.12, England.

A genetical linkage study was therefore carried out:

(1) to investigate further whether G-6-PD deficiency is *X*-linked;

(2) if so, to determine the recombination fraction between the loci for G-6-PD deficiency and colour-blindness; and

(3) to see if the difference between the expression of G-6-PD deficiency in Negroes and Caucasians is explicable on the basis of different ethnic groups.

Colour-blindness was used as the marker locus because of its well-established *X*-linkage, its frequency and the ease with which it can be tested.

Information on recombination is provided by the phenotypes of the sons of doubly heterozygous females who can be ascertained by demonstrating the presence of both traits among their sons. Whether the two traits are in the coupling or repulsion phase in the mother can be inferred from the phenotype of the maternal grandfather. It would be inefficient to ascertain the mothers through doubly affected males only. For example, if the two traits under consideration turned out to be closely linked, then all the children of mothers in repulsion would be missed except some of those in whom a cross-over had occurred. Conversely, sibships containing one or more cross-overs might be missed if the tested boy was affected by only one of the traits, even though his brothers might be doubly affected. If it be assumed that the gene frequencies for colour-blindness and G-6-PD deficiency in United States Negroes are 0·03 and 0·1, respectively, and the average male sibship size is 2, then one would expect on the average, to find about twelve doubly affected sibships per 1000 in a fully ascertained sample.

PRESENT INVESTIGATION

All the American Negro boys in ten schools were tested for colour-blindness by the Ishihara plates. Of 3648 boys 134 (3·67 %) were found to be colour-blind. Thirteen of these were lost to the follow up. Of the 121 remaining boys, 15 were found to have colour-blind brothers detected in the same survey, leaving 106 unrelated colour-blind boys. These 106 and their brothers, totalling 238, were screened for G-6-PD activity by Motulsky's (1960) dye method (Figs. 1, 2). Fifteen boys with enzyme deficiency were detected in ten families. (The allele frequency is estimated approximately as 15/238 = 0·063 as recorded in Table 1.)

Table 1. *Allele frequencies obtained from the data*

	Mutant	Normal
Protan types of colour-blindness	0·0092	0·9908
Deutan types of colour-blindness	0·0276	0·9724
G-6-PD deficiency	0·063	0·937

As it is not possible to detect heterozygotes for colour-blindness or G-6-PD deficiency (Fig. 3) with certainty, sisters of affected males were not included among the individuals studied.

Two of the ten families would not co-operate in the investigation. The members of the remaining eight families (Fig. 5) were tested for type and degree of colour-blindness by five tests (Ishihara, Tokyo Medical College Colour Vision, Hardy–Rand–Rittler, American Optical–Hardy–Rand–Rittler Plates, and the Nagel anomaloscope when possible). The enzyme estimations (Figs. 3, 4) were done by a modification of the method of Glock & McClean (1953) the so-called 'derived' method (Zinkham, Lenhard & Childs, 1958), which gives a more accurate

measure of G-6-PD activity as it circumvents the contribution of 6-phosphogluconic dehydrogenase, the enzyme responsible for the next step in the hexose monophosphate shunt metabolic pathway.

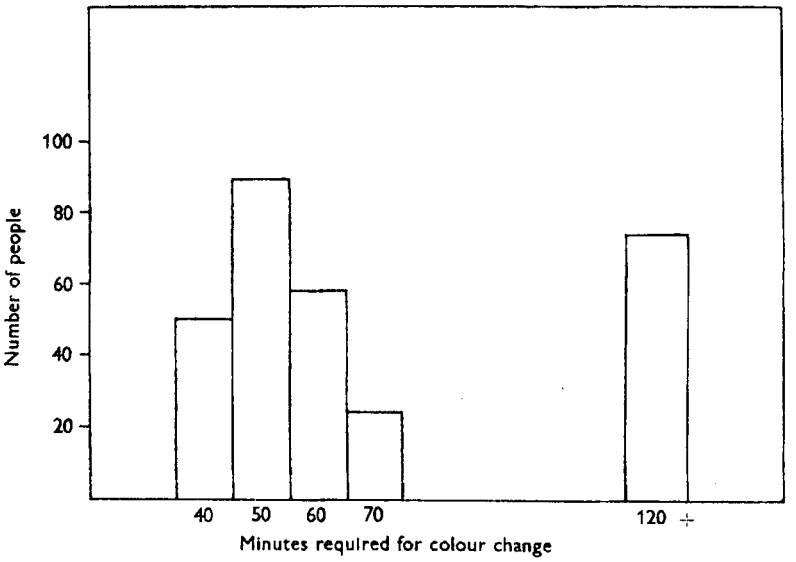

Fig. 1. Dye test for G-6-PD deficiency in 296 Negro males.

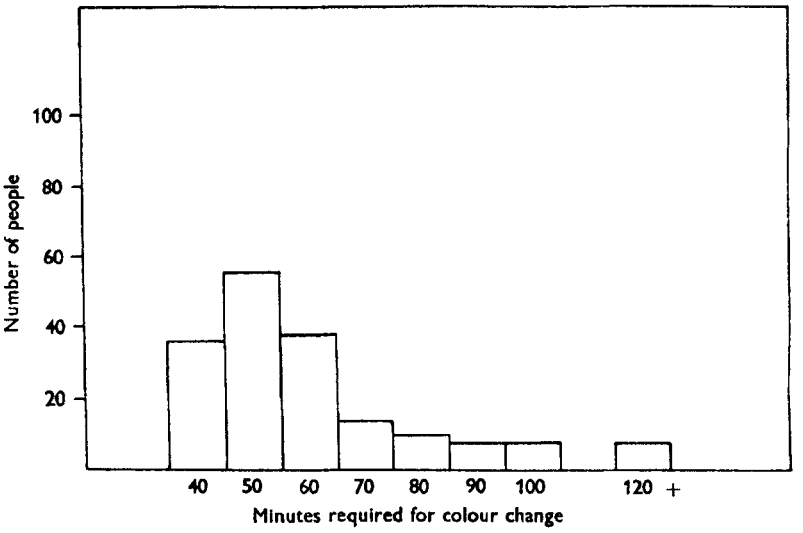

Fig. 2. Dye test for G-6-PD deficiency in 177 Negro females.

Figs. 1, 2. The dye changes colour within 40–70 min. if G-6-PD activity is normal; 2 or more hours are required if the erythrocytes are enzyme deficient. Intermediate levels (70–120) are only present in females. The histograms include all the results from the present study and others obtained during the same period.

The results of these tests are given in Appendix 1. Extensive blood grouping was done on as many of the relevant members as possible, and the blood groups are consistent with the relationships shown in the pedigrees. There were four families (A, B, C and G) in which the two genes were in the coupling phase in the mothers. Two of these families had the deutan type of colour-

vision defect, one the protan. Three other families (D, E and H) with the deutan type of defect had the genes responsible for the two traits in the repulsion phase in the mothers (Table 2). Family F gives little linkage information, as can be seen from Table 3, because the coupling phase in the pertinent mothers is unknown.

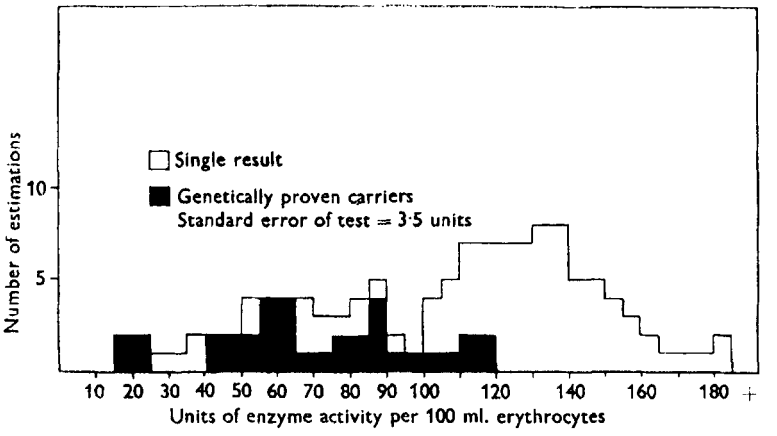

Fig. 3. Distribution of G-6-PD activity in erythrocytes of 70 Negro females.

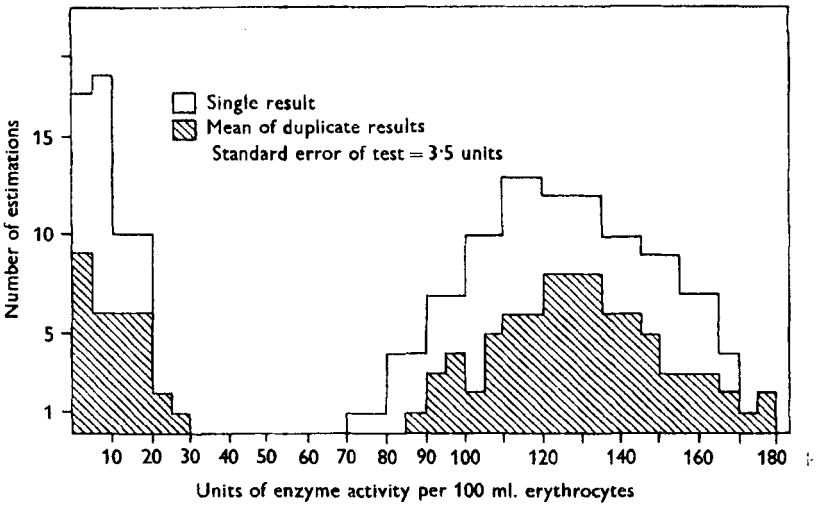

Fig. 4. Distribution of G-6-PD activity in erythrocytes of 114 Negro males.

Figs. 3, 4. Values below 30 units indicate enzyme deficiency, above 70, normal enzyme activity. Some females show intermediate values; note, however, that the 20 genetically proven carriers are not limited to this range but overlap both the deficient and normal. The histograms include all the results from the present study and others obtained during the same period.

ANALYSIS OF DATA

The method of choice for extracting linkage information on the relatively scant data available in man is the backward odds method suggested by Haldane & Smith (1947). The method consists in comparing the probability of obtaining the family data if the two loci are linked with that if they are not. The ratio of these two probabilities gives the odds for linkage. For each family

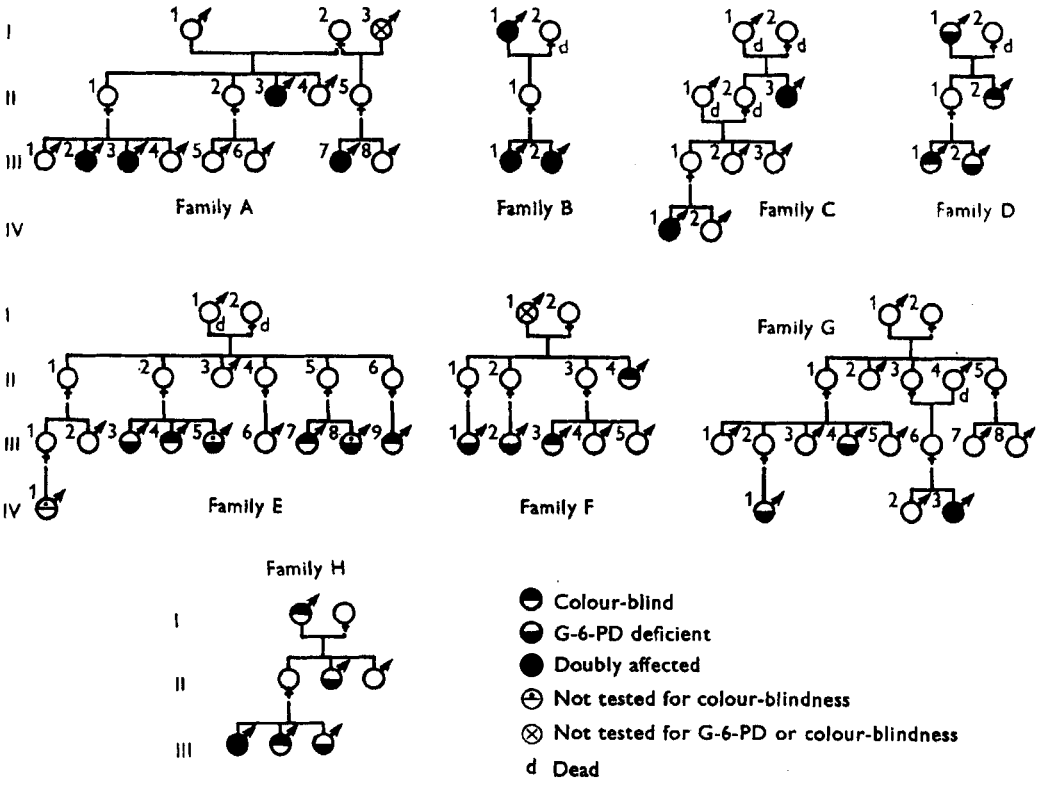

Fig. 5. Pedigrees.

Table 2. *Probable linkage phase, approximate number of recombinants, and type of colour-blindness in the families studied*

Family	Coupling	Repulsion	Number of recombinants	Type of colour-blindness in families
A	I. 2, II. 1, II. 5	—	0	Deutan
B	II. 1	—	0	Deutan
C	I. 2, II. 2, III. 1	—	0	Protan
D	—	II. 1	0	Deutan
E	—	II. 2, II. 5	0	Deutan
F	—	—	? 0	Protan
G	III. 6	—	? 1	Deutan
H	—	II. 1	1	Deutan

Table 3. *Lod scores for the G-6-PD deficiency and protan loci*

Family	0·5	0·4	0·3	0·2	0·1	0·05	0·00001
C	0	—	—	—	1·2393	1·3956	1·5468
F	0	—	—	—	0·073246	0·14068	0·22141
Total	0	0·27*	0·62*	1·04*	1·3125	1·5362	1·7682

* Lod scores interpolated from the lod curve.

the probability ratio (P_R) is calculated for a number of trial values of θ, the recombination fraction, denoted generically by θ_i, so that

$$P_R = \frac{\Pr\text{ (family, given } \theta = \theta_i)}{\Pr\text{ (family, given } \theta = 0{\cdot}5)}.$$

Since the joint probability ratio, for several independent families, is the product of the probability ratios, it is convenient to convert the ratios into logarithms and add them. The logarithm of the probability ratio is called the log of the odds or the lod score, z, and

$$z = \log_{10} \frac{\Pr\text{ (sample, given } \theta = \theta_i)}{\Pr\text{ (sample, given } \theta = 0{\cdot}5)}.$$

Since the lod is a monotonically increasing function of the probability ratio, the same value of $\hat{\theta}$ maximizes both functions. The maximum likelihood estimate of θ is found by equating the derivative of the lod function to zero and solving for $\hat{\theta}$. The estimate of the variance of the maximum likelihood estimate is given by

$$-2{\cdot}302 \left(\frac{d\theta^2}{d^2z}\right)^{-1}$$

evaluated at $\hat{\theta}$.

This method is simple in principle but tedious in practice, particularly in families with more than two generations and with complicated relationships. Smith (1953) suggested that digital computers might simplify the situation, and Simpson (1958) proved the feasibility of the suggestion. Renwick & Schulze (1961) accordingly wrote a general programme for the IBM 7090 computer, which is the one used in this study.

The programme is composed of:

(1) An elimination round, which on the basis of phenotypes fed in, determines all genotypic possibilities for each person and eliminates genotypes which are inconsistent.

(2) A probability round, in which the probability that the genes segregate in the manner observed is found for a series of trial values of θ_i. The results are expressed as lod values and constitute part of the output of the machine. Where several pedigrees are available, the lod scores obtained for each pedigree are added together.

(3) Finally, curve fitting, in which, as an approximation, a simple but flexible function is fitted by the method of least squares to the plot of lod scores against corresponding values of θ. This simplified curve is then treated as the likelihood function. The maximum point is found, and the variance of the estimate is computed by evaluating the second derivative of the function at the maximizing value of θ_i.

In order to avoid mistakes by the machine a number of internal checks exist which stop the computer in the event of any logical absurdities. The programme has been checked by comparing the results of the machine with several sets of results published by reputable hands, and the agreement has been excellent except where the published calculation has been shown to be in error. In order to avert the effects of human errors in the various stages of processing the data all steps were duplicated independently.

ASCERTAINMENT CORRECTION

The ascertainment in this study was not complete and therefore there must be as usual an 'ascertainment correction' added to the lod score (Morton, 1955). The reason for this is as follows. Suppose that in one particular family we find that the parents are of genotypes

$GgTt \times GT$, where g, t denote alleles for colour-blindness and G-6-PD deficiency respectively, and G, T are their normal alleles. The method of calculation outlined above gives a score 'z' which is the logarithm of the ratio of probabilities of observing a family like the one actually observed; but these probabilities are calculated on the assumption that the parents' genotypes are given, i.e. they are the probabilities of occurrence of such a family among all families which could arise from the given parents. Some of these families would, however, not be included in the sample if they had occurred, because they would not have qualified for the conditions of inclusion; and the probabilities which are relevant are those calculated from the set of all families which might occur and be ascertained. If f denotes such a family, we have that the probability of its occurrence among the set of all families which might be ascertained is

$$\Pr(f|\text{asc.}) = \Pr(f)/\Pr(\text{asc.}), \tag{1}$$

where $\Pr(f)$ denotes the general probability of the family f, given the parents' genotypes, and $\Pr(\text{asc.})$ similarly denotes the probability of ascertainment. Hence the correct lod score, or logarithms of the probability ratio, is

$$\begin{aligned}\log \frac{\Pr(f|\text{asc.}, \theta_i)}{\Pr(f|\text{asc.}, \tfrac{1}{2})} &= \log \frac{\Pr(f|\theta_i)}{\Pr(f|\tfrac{1}{2})} \log \frac{\Pr(\text{asc.}|\theta_i)}{\Pr(\text{asc.}|\tfrac{1}{2})} \\ &= z(f) + c(f), \end{aligned} \tag{2}$$

where $z(f)$ is the z-score, calculated for the family f on the assumption that the parents' genotypes are known, and $c(f)$ is an 'ascertainment correction'. The total score is therefore the sum of all the $z(f)$ and $c(f)$ for the different families.

We now find the value of the ascertainment correction on the following basis: that there are s male sibs in the family altogether, of which n are at school and $s-n$ at home. Of the sibs at school, at least one is colour-blind; and among all s male sibs, at least one is G-6-PD deficient.

Suppose that of the n sibs at school, $n_1 \geqslant 1$ have been found to be colour-blind. Subject to this, the probability of *non*-ascertainment is the probability that all the sibs are G-6-PD normal. For the $s-n$ sibs at home, this probability is $(\frac{1}{2})^{s-n}$. The n sibs at school are divided into n_1 colour-blind and $n-n_1$ of normal vision. If the mother is in coupling, so that the parental mating is $GT/gt \times GT$, the probability that all these sibs are G-6-PD normal is $\theta^{n_1}(1-\theta)^{n-n_1}$, whereas if the mother is in repulsion the probability is $(1-\theta)^{n_1}\theta^{n-n_1}$. Since coupling and repulsion are equally likely, at least in the data presented here, the total probability of non-ascertainment, given n_1, is

$$\Pr(\text{non-asc.}|n_1) = (\tfrac{1}{2})^{s-n}[\tfrac{1}{2}\theta^{n_1}(1-\theta^{n-n_1} + \tfrac{1}{2}(1-\theta)^{n_1}\theta^{n-n_1}]. \tag{3}$$

The probability of ascertainment, given n_1, is accordingly $1 - \Pr(\text{non-asc.}|n_1)$. We now average this over all relevant values of n_1, i.e. over $1 \leqslant n_1 \leqslant n$. Since the probability of finding n_1 colour-blind sibs among the n at school (given the parents' genotypes) is

$$\binom{n}{n_1} (\tfrac{1}{2})^{n_1} (\tfrac{1}{2})^{n-n_1} = \binom{n}{n_1} (\tfrac{1}{2})^{n},$$

the total probability of ascertainment given the parents' genotypes is

$$\begin{aligned}\Pr(\text{asc.}) &= \sum_{n_1=1}^{n} \binom{n}{n_1} [1 - (\tfrac{1}{2})^{s-n}\{\tfrac{1}{2}\theta^{n_1}(1-\theta)^{n-n_1} + \tfrac{1}{2}(1-\theta)^{n_1}\theta^{n-n_1}\}] (\tfrac{1}{2})^{n} \\ &= 2^{-s}[2^s - 1 - 2^{s-n} + \tfrac{1}{2}\{\theta^n + (1-\theta)^n\}]. \end{aligned} \tag{4}$$

Hence, from (2), we find the ascertainment correction for the family to be

$$c(f) = \log \frac{2^s - 1 - 2^{s-n} + (\frac{1}{2})^n}{2^s - 1 - 2^{s-n} + \frac{1}{2}\{\theta^n + (1-\theta)^n\}} = c_1'(f). \quad (5)$$

Note that if $s = n$, then $c(f) = c_1$ (tabulated by Morton, 1955), while if $n = 1$ then the correction is zero.

Family *F* was ascertained aberrantly through a colour-blind boy and his G-6-PD deficient cousin. The correction is assumed to be very small and has been ignored.

The relevant data on s and n are given in Tables (4, 6) for the other families.

Table 4. *Details of sibships and of ascertainment corrections*

Family ...	A	B	C	D	E	F	G	H
No. of male sibs (s)	4	2	2	2	3	—	2	3
No. of male sibs examined at school (n)	2	1	1	1	2	—	2	2
No. of colour-blind male sibs examined at school (n_1)	1	1	1	1	1	1	1	2
No. of G-6-PD deficient male sibs examined at home (n_2)	2	2	1	1	2	—	1	2
No. of G-6-PD deficient male sibs seen at school (n_3)	1	2	1	1	1	—	1	1
Type of correction	c_1'	0	0	0	c_1'	0	c_1'	c_1'

Table 5. *Lod scores for the G-6-PD deficiency and deutan loci*

Family	0·5	0·2	0·15	0·1	0·05	0·01	0·001
A	0	1·8227	2·1060	2·3751	2·6312	2·8276	2·8708
B	0	0·40824	0·46090	0·51055	0·55751	0·59333	0·60119
D	0	0·41272	0·46698	0·51847	0·56751	0·60516	0·61346
E	0	0·25841	0·31372	0·36696	0·41671	0·45339	0·46124
G	0	−0·13985	−0·13997	−0·14665	−0·17132	−0·21484	−0·22883
H	0	0·0092041	−0·063477	−0·19038	−0·44497	−1·1086	−2·1008
Ascertainment correction	0	−0·03522	−0·04846	−0·06201	−0·07801	−0·09204	−0·09571
Total	0	2·7362	3·0957	3·3720	3·4787	3·0640	2·1213

RESULTS

As it is as yet uncertain whether deutan and protan types of colour-blindness are allelic or controlled by close, but separate loci (McKusick, 1962) the data were analysed separately for the two types.

The gene frequencies, which the computer used where necessary, were calculated from the data (Table 1). For the deutan data, the average height of the probability ratio curve is 1000 (Fig. 6). Thus (from these data alone) the odds in favour of the linkage are approximately 1000 to 1 (1 being the probability ratio at 0·5). A polynomial was fitted to the lod score values (Table 5), and the maximum likelihood estimate of $\theta(\hat{\theta})$ is found to be 0·05 (Fig. 7). The second derivative of the natural logarithm of the probability curve, after normalization by a suitable transformation evaluated at the maximum likelihood point, gives I, the information, and the standard error of the estimate is $I^{-\frac{1}{2}}$ from which 90% confidence limits of 0·009–0·18 were derived.

Table 6. *Subdivision of sibships according to phenotypes and place of testing*

Parental genotype	Ascertained sibships	Number of male sibs seen at school (total n)				Number of male sibs seen at home if $n_1 > 0$ (total $s-n$)				Total male sibs
		Normal colour vision	Colour-blind	G-6-PD normal	G-6-PD deficient	Normal colour vision	Colour-blind	G-6-PD normal	G-6-PD deficient	
$GgTt \times GT$	Sibship type A	$n-n_1$	$n_1 > 0$	—	—	—	$s-n$	—	—	s
	$(n_3 = 0, n_2 > 0)$	—	—	n	$n_3 = 0$	—	—	$s-n-n_2$	$n_2 > 0$	s
$GgTt \times GT$	Sibship type B	$n-n_1$	$n_1 > 0$	—	—	—	$s-n$	—		s
	$(n_3 > 0)$	—	—	$n-n_3$	$n_3 > 0$	—	—	$s-n$		s

g—is a colour-blindness allele. t—is a G-6-PD deficiency allele.

For the protan data $\hat{\theta} = 0$. From the lod curve, lod scores (Table 3) were interpolated for θ values of 0·2, 0·3 and 0·4. A curve was fitted to the antilog of these and other lod values. The area under this curve from $\theta = 0$ to $\theta = 0{\cdot}5$ could then be considered to represent the total probability of the true value of θ lying in this range [$P(0 \leqslant \theta \leqslant 0{\cdot}5) = 1$]. A value of θ was found (0·26) which divided the area under the curve in the ratio 9:1. On the assumption that any value of θ in this range was originally equally likely, this value can be taken as the upper 90% confidence limit (Haldane & Smith, 1947).

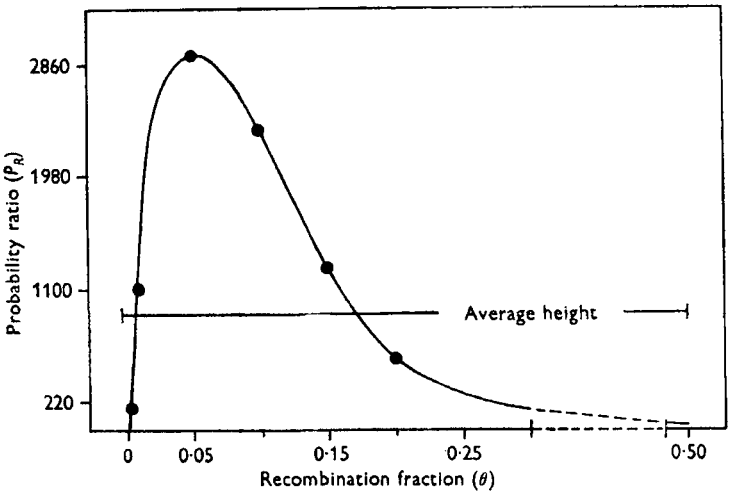

Fig. 6. $P_R = [P(\text{sample}|\theta = \theta_t)]/[P(\text{sample}|\theta = 0{\cdot}5)]$.

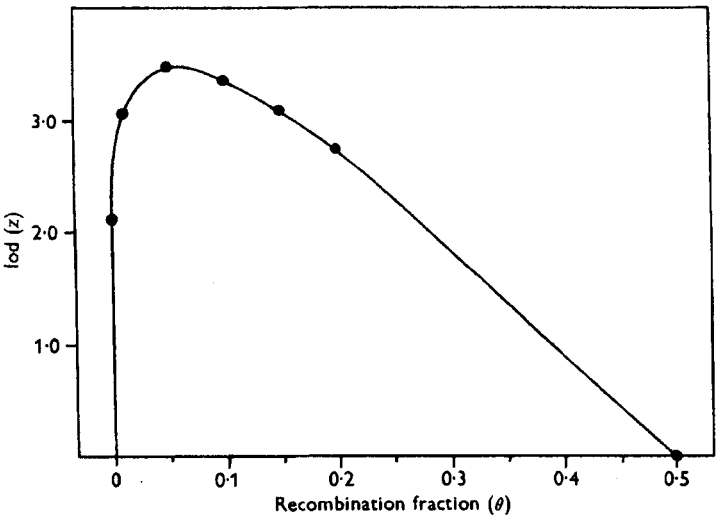

Fig. 7. $z = \log_{10}[P(\text{sample}|\theta = \theta_t)]/[P(\text{sample}|\theta = 0{\cdot}5)]$.

COMPARISON WITH OTHER STUDIES

Adam (1961*a*, *b*) reported nineteen families from Israel with no apparent recombinants, indicating close linkage between the two traits. Unfortunately, a large ascertainment correction has been ignored. An interesting finding was the high proportion of families with the two genes in repulsion (sixteen families). A possible explanation is the mixing of a long settled population

of Jews having a high frequency of G-6-PD deficiency and low frequency of colour-blindness, with an immigrant population of Jews having virtually no G-6-PD deficiency and a higher incidence of colour-blindness. A negative association between the two traits would be expected to exist for many generations because the linkage of the two traits is close and because recombination between sex-linked loci can only occur in females.

The study reported by Siniscalco, Bernini, Latte & Motulsky (1961) was carried out in Sardinia and based on four large kindred ('twelve families'). A rough estimate of θ was 0·05 (Siniscalco, Bernini & Latte, 1961). In contrast to Adam's study, the coupling phase predominated. On this evidence, and on their observation that there appears to be a positive correlation between colour-blindness and the past prevalence of malaria and therefore with the enzyme deficiency, the authors cite this as a possible example of the selection of a neutral gene through its close linkage with a highly adaptive one. However, the predominance of families with the coupling phase might arise by chance. Even in the absence of any selective advantage, if two genes are on the same chromosome and separated by a distance corresponding to a θ value of 0·05, it would take as many as sixty generations or so to reach within 5 % of equilibrium for the coupling phase. This figure of sixty generations for X-linked loci is derived by doubling the figure given by Falconer (1960) for autosomal loci.

In our study the proportions of families in repulsion and in coupling are about the same (Table 2), which is what one might expect for two loci in equilibrium.

On the linkage studies done in the three different populations between G-6-PD deficiency and colour-blindness, there is thus agreement on the presence of close linkage. The recombination estimates are all sufficiently close to assume, at least provisionally, a single G-6-PD deficiency locus.

DISCUSSION

Linkage analysis is now of practical value in man and with the assistance of electronic computers, the analysis of data has become more efficient and less laborious.

Linkage can be used to recognize genetic heterogeneity of clinical conditions as has been done with the elliptocytoses (Lawler, 1954; Morton, 1956) and the haemophilias (Graham, 1962). When sufficient common traits have been discovered, linkage may be of use in detecting carriers as trace may be kept of one gene by studying the movement of another.

Boyer, Porter & Weilbacher (1962) have recently detected qualitative variation of G-6-PD in American and African Negroes by starch gel electrophoresis. The attempt to elucidate the relationship between this new genetic polymorphism and G-6-PD deficiency was facilitated by having established with certainty that the latter is X-linked.

Most of the X-linkage estimates are based on too few observations to give anything but a rough idea of the recombination fractions.

However, with continued discovery of common traits suitable for use as markers, such as the X-linked blood group antigen Xg^a, which has a gene frequency of approximately 0·60 (Mann, Cahan, Gelb, Fisher, Hamber, Trippett, Sanger & Race, 1962), there is imminent possibility that many of the 60 known loci on the X chromosome listed by McKusick (1962) may soon be adequately mapped.

CONCLUSIONS

In the American Negro the maximum likelihood estimate of the recombination fraction for deutan colour-blindness and G-6-PD deficiency is 0·05 with 90 % confidence limits of 0·009 and 0·18. For protan colour-blindness and G-6-PD deficiency, it is zero, with 90 % confidence limit of < 0·26.

Comparisons between this study and similar studies in other races provide no evidence that more than one *X*-linked locus is responsible for G-6-PD deficiency.

The data are inadequate to provide a useful estimate of the protan–deutan map distance.

The electronic computer (IBM 7090) has proved useful in the analysis of genetical linkage based on lod scores.

We are greatly indebted to Drs J. H. Renwick, Glasgow; C. A. B. Smith, London; Louise L. Sloan, Baltimore; and W. H. Zinkham, Baltimore, for their advice and help; and to Dr F. H. Allen, Jr., Boston, for performing extensive genotype studies.

We are grateful to the IBM and the Martin Companies for the use of the IBM 7090 computer.

These studies were supported by U.S.P.H.S. grant RG-6642. The senior author is in receipt of a Wellcome Trust travelling grant.

REFERENCES

ADAM, A. (1961*a*). Linkage between deficiency of glucose-6-phosphate dehydrogenase and colour-blindness. *Nature, Lond.*, **189**, 686.

ADAM, A. (1961*b*). Linkage between enzyme deficiency of erythrocytes and colour-blindness. 2nd Int. Conf. Hum. Genet., Rome. *Int. Congr. Series*, no. 32, *Excerpta med.* E53 (abstract).

BOYER, S. H., PORTER, I. H. & WEILBACHER, R. (1962). Electrophoretic variants of glucose-6-phosphate dehydrogenase in Man. *Proc. nat. Acad. Sci., Wash.* (In the Press.)

CARSON, P. E., FLANAGAN, C. L., ICKES, C. E. & ALVING, A. S. (1956). Enzymatic deficiency in primaquine-sensitive erythrocytes. *Science*, **124**, 484–5.

CHILDS, B., ZINKHAM, W. H., BROWNE, E. A., KIMBO, E. L. & TORBERT, J. W. (1958). A genetic study of a defect in glutathione metabolism of the erythrocyte. *Johns Hopk. Hosp. Rep.* **102**, 21–37.

DERN, R. J., WEINSTEN, I. M., LE ROY, G. V., TALMAGE, D. W. & ALVING, A. S. (1954). The hemolytic effect of primaquine. I. The localization of the drug-induced hemolytic defect in primaquine-sensitive individuals. *J. Lab. Clin. Med.* **43**, 303–9.

FALCONER, D. S. (1960). *Introduction to Quantitative Genetics*, p. 21. New York: Ronald Press Company.

GARTLER, S. M., VULLO, C. & GARDINI, E. (1962). Glucose-6-phosphate dehydrogenase deficiency in an *XO* individual. *Cytogenetics* (in the Press).

GLOCK, G. E. & MCLEAN, P. (1953). Further studies on the properties and assay of glucose-6-phosphate dehydrogenase and 6-phospho-gluconate dehydrogenase of rat liver. *Biochem. J.* **55**, 400–8.

GRAHAM, J. P. (1962). *Amer. J. Hum. Genet.* (in the Press).

HALDANE, J. B. S. & SMITH, C. A. B. (1947). A new estimate of linkage between the genes for colour-blindness and haemophilia in Man. *Ann. Eugen., Lond.*, **14**, 10–31.

HOCKWALD, R. S., ARNOLD, J., CLAYMAN, C. B. & ALVING, A. S. (1952). Status of primaquine. 4. Toxicity of primaquine in negroes. *J. Amer. Med. Ass.* **149**, 1568–70.

LAWLER, S. D. (1954). Family studies showing linkage between elliptocytosis and the Rhesus blood group system. *Caryologia*, Suppl. **6**, 26.

MCKUSICK, V. A. (1962). On the *X* chromosome of Man. *Medicine* (in the Press).

MANIFOLD, J. A. (1931). Report on a trial of plasmaquine and quinine in the treatment of benign tertian malaria. *J. R. Army Med. Cps*, **56**, 321–38.

MANN, J. D., CAHAN, A., GELB, A. G., FISHER, N., HAMBER, J., TRIPPETT, P., SANGER, R. & RACE, R. R. (1962). A sex-linked blood group. *Lancet*, **1**, 8–10.

MARKS, P. A. (1959). *Genetics. Genetic Information and the Control of Protein Structure and Function*, p. 199. Ed. H. E. Sutton. New York: The Josiah Macy, Jr. Foundation.

MARKS, P. M. & GROSS, R. T. (1959). Erythrocyte glucose-6-phosphate dehydrogenase deficiency: Evidence of differences between Negroes and Caucasians with respect to this genetically determined trait. *J. Clin. Invest.* **38**, 2253–62.

MORTON, N. E. (1955). Sequential tests for the detection of linkage. *Amer. J. Hum. Genet.* **7**, 277–318.
MORTON, N. E. (1956). The detection of linkage between the genes for elliptocytosis and Rh blood type. *Amer. J. Hum. Genet.* **8**, 80–96.
MOTULSKY, A. G. (1960). Population genetics of glucose-6-phosphate dehydrogenase deficiency of the red cell, in *Proceedings of the Conference on Genetic Polymorphisms and Geographic Variations in Disease*, p. 270. Ed. B. S. Blumberg. U.S. Department of Health, Education and Welfare. National Institutes of Health, Bethesda 14, Maryland.
NILSSON, I. M., BERGMAN, S., RAITALU, J. & WALDENSTRÖM, J. (1959). Haemophilia in a 'girl' with male sex-chromatin pattern. *Lancet*, **1**, 264–66.
PENROSE, L. S. (1961). *Recent Advances in Human Genetics*, p. 134. Edited by L. S. Penrose. London: J. and A. Churchill Ltd.
POLANI, P. E., LESSOF, M. H. & BISHOP, P. M. F. (1956). Colour-blindness in 'ovarian agenesis' (gonadal dysplasia). *Lancet*, **2**, 120.
RENWICK, J. H. & SCHULZE, J. (1961). A computer programme for the processing of linkage data from large pedigrees. 2nd int. Conf. Hum. Genet., Rome. *Int. Congr. Series*, no. 32, *Excerpta med.* E145 (abstract).
SIMPSON, H. R. (1958). The estimation of linkage on an electronic computer. *Ann. Hum. Genet., Lond.*, **22**, 356–61.
SINISCALCO, M., BERNINI, L. & LATTE, B. (1961). Linkage between G-6-PD deficiency, colour-blindness and haemophilia. 2nd Int. Conf. Hum. Genet., Rome. *Int. Congr. series*, no. 32, *Excerpta med.* E53 (abstract).
SINISCALCO, M., BERNINI, B., LATTE, B. & MOTULSKY, A. G. (1961). Favism and thalassaemia in Sardinia and their relationship to Malaria. *Nature, Lond.*, **190**, 1179–80.
SMITH, C. A. B. (1953). The detection of linkage in human genetics. *J. Roy. Statist. Soc.* B, **15**, 153–92.
TURCHETTI, A. (1948). Forme poco frequenti di emoglobiniuria da farmaci in corco di infezione malarica. *Rif. med.* **62**, 325.
ZINKHAM, W. H., LENHARD, R. E. & CHILDS, B. (1958). A deficiency of glucose-6-phosphate dehydrogenase activity in erythrocytes from patients with favism. *Bull. Johns Hopk. Hosp.* **102**, 169–75.

APPENDIX 1

Pedigree no.	Parent or parents	Sex	Age (years)	Colour-blindness	G-6-PD activity in units of enzyme per 100 ml. RBC	Motulsky's test. Minutes required for colour change
				Family A		
I. 1	—	M	37	—	103·8	50
					105·5	50
I. 2	—	F	43	—	89·9	70
					95·5	60
II. 1	I. 1, 2	F	23	—	43·6	120
					45·8	110
II. 2	—	F	20	—	77·7	80
					80·2	80
II. 3	—	M	17	Deutan,	2·3	> 120
				grade 1	2·4	> 120
II. 4	—	M	15	—	153·7	40
					144·9	40
II. 5	I. 2, 3	F	22	—	60·1	80
					62·3	80
III. 1	II. 1	M	9	—	137·7	50
					138·9	50
III. 2	—	M	8	Deutan,	24·2	> 120
				grade 2	25·3	> 120
III. 3	—	M	7	Deutan,	15·5	> 120
				grade 2	19·9	> 120
III. 4	—	M	4	—	155·6	40
					140·5	40
III. 5	II. 2	M	5	—	122·4	50
					112·4	50
III. 6	—	M	4	—	91·2	70
					85·9	70
III. 7	II. 5	M	7	Deutan	3·4	> 120
					2·2	> 120

APPENDIX 1 (*continued*)

Pedigree no.	Parent or parents	Sex	Age (years)	Colour-blindness	G-6-PD activity in units of enzyme per 100 ml. RBC	Motulsky's test. Minutes required for colour change
				Family A (cont.)		
III. 8	—	M	6	—	107·8	50
					103·4	50
				Family B		
I. 1	—	M	73	Deutan,	5·4	> 120
				grade 3	6·2	> 120
II. 1	I. 1, 2	F	40	—	—	80
						80
III. 1	II. 1	M	12	Deutan,	8·4	> 120
				grade 3	7·2	> 120
III. 2	—	M	6	Deutan	4·6	> 120
					5·3	> 120
				Family C		
II. 3	I. 1, 2	M	57	Protan,	18·6	> 120
				grade 3	16·8	
III. 1	II, 1, 2	F	39	—	181·4	40
					188·4	
III. 2	—	M	37	—	122·4	50
					116·4	
III. 3	—	M	28	—	107·9	60
					119·3	
IV. 1	III. 1	M	12	Protan,	14·7	> 120
				grade 3	15·2	
IV. 2	—	M	11	—	124·6	50
					120·8	
				Family D		
I. 1	—	M	65	—	3·6	> 120
					4·0	> 120
II. 1	I. 1, 2	F	38	—	80·7	60
					85·5	60
II. 2	—	M	36	Deutan,	130·2	40
				grade 3	128·9	40
JII. 1	II. 1	M	10	Deutan,	123·7	60
				grade 3	118·2	60
III. 2	—	M	7	—	5·7	> 120
					4·6	> 120
				Family E		
II. 1	I. 1, 2	F	43	—	121·7	60
					128·8	60
II. 2	—	F	35	—	59·4	90
					58·2	90
II. 3	—	M	29	—	150·0	60
					135·5	60
II. 4	—	F	24	—	118·8	50
					114·4	50
II. 5	—	F	22	—	76·0	40
					89·0	40
II. 6	—	F	19	—	71·6	50
					68·1	50
III. 1	II. 1	F	25	—	—	50
						50
III. 2	—	M	24	—	—	40
						40
III. 3	II. 2	M	12	—	2·3	120
					2·4	120

APPENDIX 1 (*continued*)

Pedigree no.	Parent or parents	Sex	Age (years)	Colour-blindness	G-6-PD activity in units of enzyme per 100 ml. RBC	Motulsky's test. Minutes required for colour change
				Family E (cont.)		
III. 4	—	M	8	Deutan	135·7	50
					142·6	40
III. 5	—	M	1	?	—	> 120
						> 120
III. 6	II. 4	M	8	—	—	60
						50
III. 7	II. 5	M	4	Deutan	—	40
						40
III. 8	—	M	1	?	—	> 120
						> 120
III. 9	II. 6	M	5	Deutan	—	60
						60
IV. 1	III. 1	M	3	?	—	50
						50
				Family F		
I. 1	—	M	54	Unco-operative	—	—
I. 2	—	F	52	—	—	50
						50
II. 1	I. 1, 2	F	33	—	19·1	100
					18·5	100
II. 2	—	F	31	—	52·6	110
					51·4	100
II. 3	—	F	30	—	65·8	70
					66·9	70
II. 4	—	M	28	Protan, grade 3	132·4	50
					130·8	
III. 1	II. 1	M	5	—	—	> 120
						> 120
III. 2	II. 2	M	8	—	—	> 120
						> 120
III. 3	II. 3	M	11	Protan	—	50
						50
III. 4	—	M	6	—	—	60
						60
III. 5	—	M	5	—	—	60
						60
				Family G		
I. 1	—	M	65	—	—	50
						50
I. 2	—	F	60	—	120·8	50
					124·6	50
II. 1	I. 1, 2	F	41	—	114·7	50
					118·2	50
II. 2	—	M	39	—	128·6	50
					129·8	
II. 3	—	F	37	—	75·9	50
					67·2	50
II. 5	—	M	26	—	116·2	50
					114·3	40
III. 1	II. 1	M	24	—	130·8	50
					136·7	40
III. 2	—	F	23	—	58·4	80
					56·9	80

APPENDIX 1 (*continued*)

Pedigree no.	Parent or parents	Sex	Age (years)	Colour-blindness	G-6-PD activity in units of enzyme per 100 ml. RBC	Motulsky's test. Minutes required for colour change
				Family G (cont.)		
III. 3	—	M	17	—	142·6	50
					138·5	50
III. 4	—	M	15	—	8·4	> 120
					6·8	> 120
III. 5	II. 1	M	13	—	160·2	40
					162·1	40
III. 6	II. 3, 4	F	24	—	117·3	40
					113·5	50
III. 7	II. 5	M	11	—	140·5	40
					147·6	40
III. 8	—	M	6	—	—	50
						50
IV. 1	III. 2	M	7	—	7·2	> 120
					7·9	> 120
IV. 2	III. 6	M	7	—	134·8; 168·1	50; 40
					148·1; 160·1	50; 40
IV. 3	—	M	5	Deutan, grade 3	8·4	> 120
					9·5	> 120
				Family H		
I. 1	—	M	57	Deutan, grade 3	134·3	50
					136·6	40
I. 2	—	F	52	—	61·2	90
					63·5	90
II. 1	I. 1, 2	F	28	—	84·8	70
					90·2	70
II. 2	—	M	26	—	4·2	> 120
					3·1	> 120
II. 3	—	M	23	—	109·2	60
					107·4	60
III. 1	II. 1	M	10	Deutan, grade 3	2·2	> 120 > 120
					3·2	> 120
III. 2	—	M	8	Deutan, grade 3	107·7	50; 60
					111·8	60
III. 3	—	M	5	—	7·6	> 120
					8·2	> 120